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Effect of seed treatment with natural products on early arbuscular mycorrhizal colonization of wheat by *Claroideoglomus claroideum*

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Summary

Commercially available natural products (NP) were applied to seed of winter wheat which was sown in a sandy soil infected with the arbuscular mycorrhizal fungus *Claroideoglomus claroideum*. The aim was to investigate whether an isoflavonoid (formononetin), different humates/algae extracts and inorganics (dolomitic lime, silicates) improved the early mycorrhization process. Experiments were carried out under controlled conditions in small pots in growth chambers. Plants were harvested between 14 and 29 days after treatment. The results showed that the isoflavonoid accelerated the mycorrhiza formation by increasing the number of mycorrhizal infection points with consequently higher infection frequency, intensity, and mycorrhized root biomass. A dolomitic limestone also improved the mycorrhizal infection process. No effects were found by the humates, extracts of algae and a silicate product. While in general the mycorrhization was most influenced by lower dose rates of NP (0.1 and 1 mg seed^{-1}), a higher rate of 10 mg seed^{-1} had lower and sometimes negative effects on the mycorrhization. On the other side, highest NP doses had positive effects on some plant growth parameter, which may have been related to the potassium content of e.g. the humate products, or because these products had plant growth promoting effects. It can not be excluded that some products, like the dolomitic limestone had an indirect effect on the mycorrhiza development via influencing other micro-organisms in the wheat rhizosphere.

Introduction

Arbuscular mycorrhizal fungi (AMF) of the Glomeromycota play important roles for plant nutrition and resilience of plants after abiotic stress situations (Smith and Read, 2008), in particular under acid soil conditions. Improved root health and higher tolerance to root pathogens and nematodes were reported, too (Whipps, 2004; Kaewchai et al., 2009). Research in various agronomic plants have shown that early, quick and extensive mycorrhizal root colonization is one of the most important pre-requisites to make use of the symbiosis for crop production (García-Garrido et al., 2009). This applies in particular for short season crops, or annual crops like cereals. Accordingly, technologies were developed to inoculate crops with arbuscular mycorrhizal fungi at time of planting. Inoculation is nothing else then placing a substrate with a high and concentrated mycorrhizal infection potential under the germinating seed of a plant. This is making sure that the first roots get immediately infected, and more and better than with the native AMF population which concentration is often low in natural soils (Sieverding, 1991; Salvioli et al., 2012).

It is well known that cereals like wheat, barley and oats are mycorrhizal, and under acidic soil conditions, like in volcanic derived Andosols of Southern Chile, AMF are important for phosphate (P) uptake of the crop and for tolerating aluminum (Al) stress conditions of these soils (Mora et al., 2006). Problematic low P soils are also found in other parts of the world and in South America, like in Argentina, Bolivia, Paraguay and Uruguay where wheat production is of primary socio-economic importance. When the mycorrhizal symbiosis is to be managed, the inoculation technology (see above: artificially increased inoculum potential under the seed) is likely not practical and not economic, considering that likely the equivalent value of 1 t grain (e.g. 150-200 USD) or more may be necessary for application of inocula per hectare, as found by own internet searches. Also, as far as we know, in the above mentioned countries no suitable commercial AMF inoculum is available. The alternative to applying AMF inocula is the management of the native AMF population by agronomic practices in such a way that the early mycorrhizal development is enhanced. This strategy was proposed longer time ago (Sieverding, 1991) and some agronomic practices, like incorporation of P fertilizers enhanced AMF in wheat (Covacevich et al., 2007). Also, selection of wheat varieties with strong early root growth appears to be a potential measure to obtain improved early mycorrhizal infection rates (Castillo et al., 2012), while some fungicide seed treatments may or may not decrease the root colonization (Burrows and Ahmed, 2007; Murillo-Williams and Pedersen, 2008). Seed treatment with chemical or biological products which improve early mycorrhizal infection would indeed be the easiest practical way to influence AMF in extensive large acreage cereal production, as farmers are adapted to treat wheat seed with pesticides anyway. It was thus the objective of the here presented research to investigate whether some selected inert natural products (NP) affect the early mycorrhizal colonization in wheat. One NP was found some 20 years ago to improve the early AM infection of plants: formononetin, an isoflavon which is extracted from clover roots (Nair et al., 1991; Tsao et al., 2006). This still patented active showed a quite consistent improvement in mycorrhization of roots and of yields (Davis et al., 2005; Westphal et al., 2008). It is used as a reference NP in our studies. Other NP are often advertised that they improve the native AMF action but research results of their effects are not available. These other natural products are either humic and or fulvic acids, extracts of algae, dolomite limestone and sodium silicates, and are all commercially available not only in Chile but also elsewhere. We investigated the effects in growth chambers at optimum temperatures using one AMF fungal species, *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler. This fungal species was isolated from cultivated soils of Southern Chile and had proven infectivity in different soils and different agronomic crops (Castillo et al., 2006; Castillo et al., 2010; Castillo et al., 2013). The results presented here are part of a series of growth chamber and field experiments with the aim to evaluate the effect of different NP, as well as micro-organisms on early AMF root colonization in different cereals species and different soils. The current paper reports the results of three sequential and related experiments to determine the effect of NP on germination of wheat seeds and early AMF root colonization parameter and growth parameter of the plant.

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Materials and methods

Soil substrate for bio-assays

Sandy soil was used in the experiments which had been sterilized in an autoclave for one hour each on two consecutive days. The sand had pH 6.7 and was low in available nutrients. The sand was mixed with 2.5% (v/v) of a soil substrate containing 57 spores per mL of the mycorrhizal fungus *Cl. claroideum*, so that the final mixture contained about 1-2 spores per mL. This is a relative low number of spores in field soils but was chosen on purpose as sub-optimal so that the NP could promote the fungus for infection. This AMF species had been multiplied on maize as host plant in a soil-perlite mixture for 6 months. The fungal species originated from a soil of the Araucanía Region in Southern Chile where it is frequently found. For the experiments the soil was added to 250 mL plastic pots.

Wheat variety

The *Triticum aestivum* L. variety "Kumpa-INIA" was selected for the experiments because this had shown low root growth and low infection rates in earlier experiments (Castillo et al., 2012). We thus expected good responses in growth parameter to the application of NP if there were any. For the experiments in soil, the seeds physiological dormancy was broken through pre-drying by heating in a furnace at 30 ºC for 7 days (ISTA, 2008). Seed had not been treated with fungicides or insecticides before use. Seed was surface sterilized with sodium-hypochlorite, washed and placed on wet filter paper to germinate. When the first signs of germination were visible, equal progressed seed was place in planting holes one in each pot. One mL of either water (control) or water with NP at specific concentrations was added onto each seed so that the seed and the soil below the seed were wetted with the NP. The seed was then tapped with the sand.

Natural products (NP)

The following NP were used:

(B) BORREGRO HA-2 Powder, company Borregaard Ligno Tech Bridgewater, NJ, USA: it is a complete water soluble potassium (K) humate product, made by extraction of high quality leonardite ore. It is 50 % humic acid and 50 % fulvic acid.

(D) DISPER Alghum GS, company DISPER, Alicante, Spain (in Chile: AM ecological, Santiago de Chile): complete water soluble product containing humic extracts from American Leonardite and seaweed extract from *Ascophyllum nodosum*.

(M) Myconate®, company Plant Health Care USA Agriculture, Pittsburgh, USA: powder product of a potassium salt water-soluble form of formononetin (7-hidroxy, 4'-metoxy isoflavone).

(N) Natural Green®, company natural green GmbH, Cologne, Germany: finely milled energized product derived from natural deposits consisting of dolomite limestone, mainly $CaCO_3$ (79.19 %) and $MgCO_3$ (4.62 %).

(P) POWHUMUS WSG-85®, company HUMINTECH GmbH, Düsseldorf, Germany: contains water soluble potassium salt of humic and fulvic acids (80-85 % w/w) from Leonardite.

(Q) Quick Sol®, company Beyond International Inc., Miami, USA (in Chile: AM ecological, Santiago de Chile): water soluble sodium silicate, contains silicon (36 %), sodium (6 %), humic and fulvic acids (each 1 %).

All products were dissolved or dispersed in water and 1 mL solution was applied per seed containing 0.1 mg (low concentration), 1 mg (normal concentration) and 10 mg (high concentration) of each product. The concentrations were derived from the use recommendation of Myconate® for optimum mycorrhizal colonization (Koide, 1999). Considering a weight of about 40 g per 1000 wheat seeds, and 100 kg of wheat seed ha^{-1}, the 2.5 x 10^6 ha^{-1} seeds will have received 0.25, 2.5 or 25 kg NP product.

Seed germination tests

Seed germination percentage were evaluated according to the rules of the International Seed Testing Association (ISTA, 2004) using four replicates of 25 seeds per treatment sowing previously above pleated sterile moistened paper; 1 mL of differently concentrated NP was added to each seed as well as control to which sterile water was added. After applying the NP on the seeds, the plates were tightly sealed with parafilm. The experiment was conducted in a semi-controlled growth chamber adjusted to 25 °C±2 °C day/night temperatures, and a 16 h photoperiod at 310 µmol m^{-2} s^{-1} of photosynthetic photon flux density. For all NP treatments, including the control, evaluations took place in accordance with ISTA (2008) at eight days after treatment. In some cases, also plumule elongation and radicle length were measured.

Mycorrhization bioassays

The experiments were conducted in a growth chamber with permanent 25 °C ± 2 °C temperature, and a 16 h photoperiod at 310 µmol m^{-2} s^{-1} of photosynthetic photon flux density. The experimental set up was a complete randomized design, with five replicates per treatment and harvest. Plants were watered daily, no fertilizer were applied. Five pots of each treatment were harvested at 14 or 15 days after planting, DAP (H1); 17 DAP (H2); 20 DAP (H3); 24 DAP (H4) and 29 DAP (H5). These harvests corresponded to plant growth stages 1.12 and 1.13 (two and three leaves unfolded) (Zadock et al., 1974). For each harvest, plant shoots were harvested and the dry weight was determined; before, the length of the longest leave was measured from the old germinated seed up to the tip. Roots were carefully washed to remove any sand adhered to the surface, length of the longest root (from germinated seed to tip) was measured and fresh weight and dry weight were determined. A subsample of fresh root material was taken randomly after the whole root sample had been cut into about 1 cm long segments. This subsample was used for determination of AM colonization. AM fungal structures in roots were stained with 0.05 % trypan blue at 60 ºC for 5 min in a water bath (Koske and Tessier, 1983) after heating in 2.5 % KOH at 60 ºC for 12 min, rinsing then in a few changes of water, and acidification in 1 % hydrochloric acid at room temperature for one hour.

Fungal colonization was evaluated according to Trouvelot et al. (1986) and expressed as frequency percentage (F%), mycorrhization intensity percentage (M%) and mycorrhizal infection entry points (EP) in root cortex. Twenty randomly chosen root fragments of 1 cm in length of each treatment were mounted on slides and examined microscopically at 100-400x magnification under an Olympus YS100 light microscopy. Frequency percentage was assessed by relating the number of infected 1 cm root segments over number of total root segments. Intensity of AM infection in the root cortex was determined by the five-class classification system following the method described by Trouvelot et al. (1986) where the numbers indicate the proportion (%) of root cortex colonized by the fungus (Alarcón and Cuenca, 2005; Castillo et al., 2012), whilst EP are the number per 20 root segments. Finally, the root biomass infected with mycorrhiza was calculated by multiplying the root fresh weight with the mean intensity of AM infection divided by 100 (Castillo et al., 2012) where the infection intensity classes were transformed to percentage scales.

Statistical analysis

The data obtained were submitted to the Shapiro-Wilk normality test and then an ANOVA of one factor was carried out using the Duncan *a posteriori* multiple range means separation test ($P \leq 0.05$). All analyses were conducted with the computer program SPSS version 12.0 (SPSS, 2003).

Results

Effect of different NP

The seed germination test showed that the 0.1 and 1 mg seed^{-1} rate had no negative effect on seed germination (Fig. 1). At 10 mg seed^{-1}, products D, M, Q decreased the germination to rates between 90 and 95 %, although not significantly.

Mycorrhizal entry points, frequency and intensity of root infection, and infected root biomass in general appeared to be highest at the lowest concentration of the NP and lowest at the highest concentrations (Fig. 2). Myconate (M) increased the mycorrhizal parameter at the lowest concentration, significantly. Frequency of root infection was also increased by D and N, at the lowest concentration. Infected root biomass was significantly increased over the control with B, D, M, N at the lowest dose rate. At the medium dose rate only M increased mycorrhizal entry points and frequency of infection, while at the high dose M and P and Q decreased the infection parameter.

Growth parameters were influenced by the NP and the dose rate used (Tab. 1). While plant length was often higher than C when D, M, N and Q where applied at the medium and high rate, B and P increased height at medium rate, only. Root length had significantly increased often with the highest dose of the NP, except with B. Shoot dry weight increased in general with higher dose of the NP. Root dry matter was also generally highest at the highest NP dose.

Effect of Myconate (M) and Natural Green (N) on mycorrhizal and plant development over time

While in the first experiment harvest of the plants was very early, the second experiment aimed to define the development of the mycorrhization over time. Two NP were selected, M as reference product and N. Natural Green had shown some intermediate effects on the mycorrhization and on the plant growth parameter in the first experiment at 15 DAP. In this experiment only one dose was tested: 1 mg NP plant^{-1}.

All mycorrhizal parameter increased over time and both NP had positive effects on the development of the mycorrhiza in roots, as shown by numbers of entry points, frequency and intensity of infection and infected root biomass (Fig. 3). Both, M and N increased the mycorrhization often significantly over the control plants, and more with more advancement over the time. However, it should be noted that the mycorrhizal infection intensity was very low in general and less than 1% of the root (Fig. 3, frequency) was infected up to 24 days after planting.

Plant growth parameter increased over time (Tab. 2). While plant height was not influenced by NP, root length, shoot dry weight and root dry weight increased through NP already from the second harvest on.

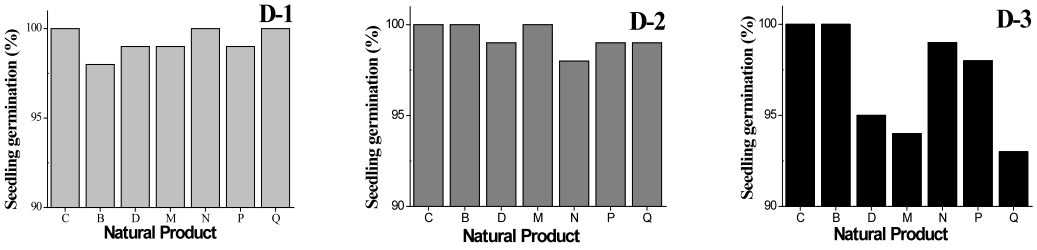

Fig. 1: Seed germination percentage of *T. aestivum* var. "Kumpa-INIA" with application of BorreGro (B), Dispher Alghum (D), Myconate (M), Natural Green (N), Pow Humus (P), Quick Sol (Q) and control without addition NP (C) at three concentrations: 0.1 mg mL^{-1} seed^{-1} (D-1); 1 mg mL^{-1} seed^{-1} (D-2), and 10 mg mL^{-1} seed^{-1} (D-3). No significant differences were found between values at each concentration using Duncan (P≤0.05).

Tab. 1: Plant parameters of *T. aestivum* var. "Kumpa-INIA" with application of BorreGro (B), Dispher Alghum (D), Myconate (M), Natural Green (N), Pow Humus (P), Quick Sol (Q), and a control (C) without addition NP, at three concentrations: 0.1 mg mL^{-1} seed^{-1} (D-1); 1 mg mL^{-1} seed^{-1} (D-2), and 10 mg mL^{-1} seed^{-1} (D-3).

Plant parameters	Doses	Natural Products						
		C*	B	D	M	N	P	Q
Height (cm)	D-1	13.5 b	18.4 ab	15.5 b	23.7 a	13.1 b	18.3 ab	17.4 b
	D-2	13.5 b	20.8 a	20.2 a	20.8 a	19.5 a	20.1 a	20.8 a
	D-3	13.5 b	18.1 ab	23.1 a	23.3 a	20.8 a	18.4 ab	19.8 a
Root lenght (cm)	D-1	16.2 b	24.2 ab	24.8 ab	31.7 a	20.8 ab	30.2 a	23.7 ab
	D-2	16.2 b	20.3 b	39.7 a	21.8 b	27.6 ab	24.6 ab	25.6 ab
	D-3	16.2 b	15.9 b	29.1 a	27.6 a	25.5 a	27.2 a	26.6 a
Shoot dry weight (mg plant^{-1})	D-1	15.3 bcd	17.7 abc	20.0 a	20.3 a	10.7 d	15.0 cd	17.3 abc
	D-2	15.3 b	20.0 ab	27.7 a	22.7 ab	19.7 ab	25.0 ab	24.7 ab
	D-3	15.3 b	30.3 a	28.3 ab	28.0 ab	23.0 ab	21.3 ab	23.0 ab
Root dry weigth (mg plant^{-1})	D-1	12.0 b	15.0 ab	19.0 a	11.7 b	16.7 ab	11.3 b	18.3 a
	D-2	12.0 c	17.7 bc	18.0 b	16.0 bc	24.7 a	18.3 b	20.7 ab
	D-3	12.0 c	29.0 ab	33.7 a	30.3 ab	21.3 bc	33.3 a	27.0 ab

In each row, for each dose, values not sharing a letter in common differ significantly according to Duncan test (P≤0.05).
*Values for C were repeated in each concentration as a reference value for the NPs.

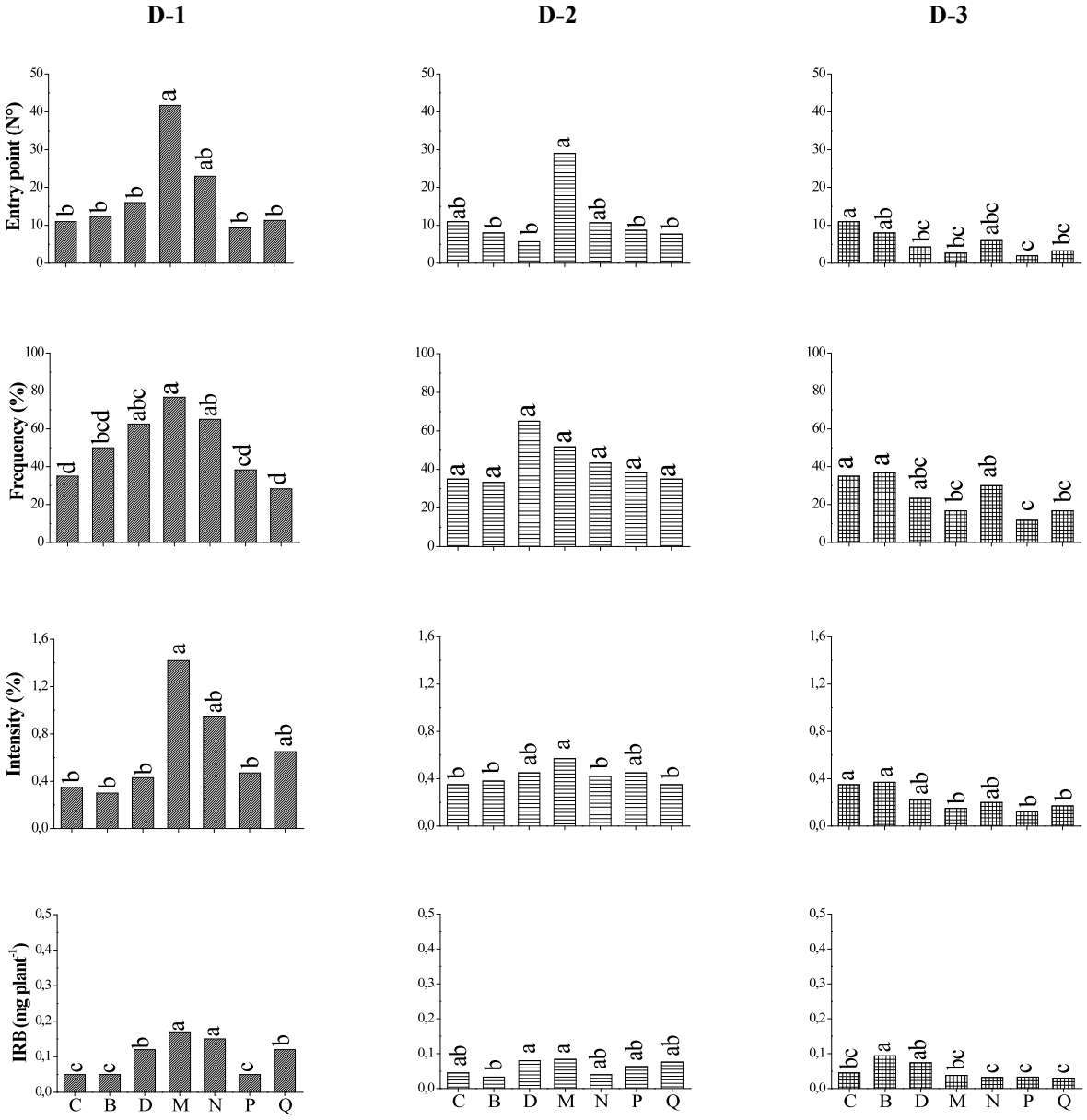

Fig. 2: Mycorrhizal colonization parameters: entry points, frequency of infection, infection intensity, and infected root biomass (IRB) in *T. aestivum* var. "Kumpa-INIA" with application of BorreGro (B), Dispher Alghum (D), Myconate (M), Natural Green (N), Pow Humus (P), Quick Sol (Q) and control without addition NP (C) at three concentrations: 0.1 mg mL^{-1} $seed^{-1}$ (D-1); 1 mg mL^{-1} $seed^{-1}$ (D-2), and 10 mg mL^{-1} $seed^{-1}$ (D-3). Columns for each concentration sharing a letter in common do not differ significantly according to Duncan test (P≤0.05).

Discussion

For this study, we took mycorrhizal infection entry points in roots as one of the main parameter because its number may be the direct result of the NP treatments on germination of fungal propagules and branching of mycorrhizal germination structures in soil. The infection intensity was quantified by conventional methods as this was a method applicable under the conditions in Chile, and because real time PCR was considered, at the time of the start of the experiments, a poor method for quantifying mycorrhizal colonization in roots (König et al., 2010).

The results of this investigation showed that NP, when used as seed treatments, can differ strongly in their effects on early mycorrhiza formation in wheat. The investigated products can be characterized into three groups: 1) potassium salts of plant extracts (M), 2) potassium salts of lignates, humates and sea weed extracts, and mixtures thereof (B, D, P), and 3) inorganics (N, Q).

Only M has been more intensively investigated and tested in different agronomic crops for mycorrhiza effects, in the past (Nair et al., 1991; Davies et al., 2005; Novais and Siqueira, 2009). The isoflavonoid formononetin stimulated AMF spore germination, hyphal branching in the soil and root colonization by AM fungi (Nair et al., 1991; Siqueira et al., 1991) and increased the number of appressoria and/or entry points to the roots of soybeans (Silva-Junior and Siqueira, 1997). In our study, entry point number of the AM fungus *Cl. claroideum* was also most increased by M in wheat roots, and was higher than with any other NP, with some exception for N. As a result of this, higher infection frequency and intensity were found. Because root dry matter production also developed better, higher root biomass was occupied with mycorrhizal structures when the wheat seed was treated. Myconate led to quicker and stronger mycorrhization of wheat as compared to no seed treatment. Assuming

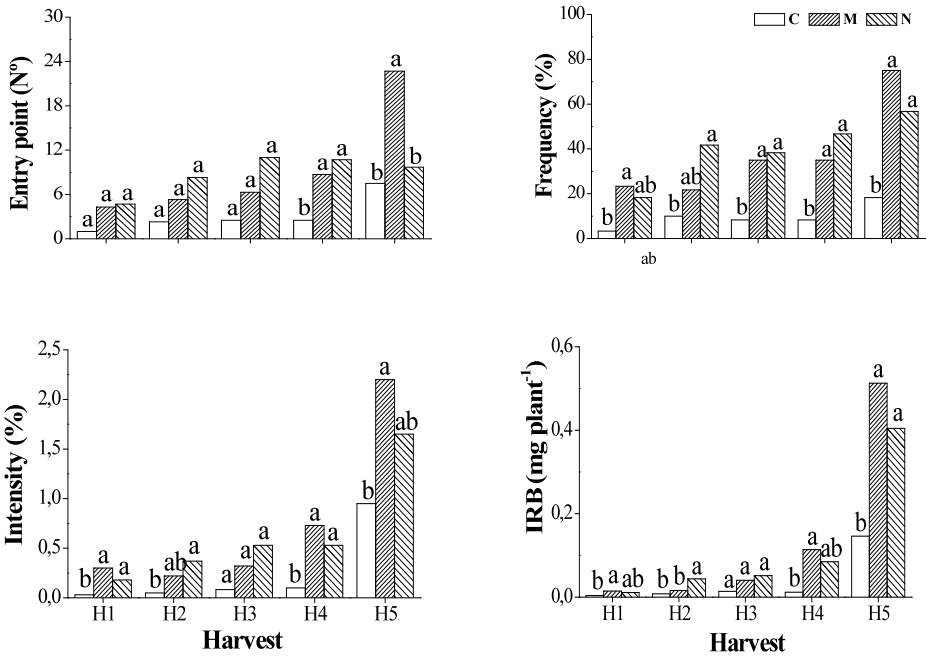

Fig. 3: Mycorrhizal colonization parameters: entry points, frequency of infection, infection intensity, and infected root biomass (IRB) in *T. aestivum* var. "Kumpa-INIA" with application of Myconate (M), Natural Green (N), and a control (C) without addition NP at 14 (H1), 17 (H2), 20 (H3), 24 (H4), and 29 (H5) days after treatment. Data of each harvest not sharing a letter in common differ significantly according to the Duncan test (P≤0.05).

Tab. 2: Plant growth parameters of *T. aestivum* var. "Kumpa-INIA" with application of Myconate (M), Natural Green (N), and a control without addition of NP (C) at 14 (H1), 17 (H2), 20 (H3), 24 (H4), and 29 (H5) days after treatment.

Plant parameters	Harvest	*Cl. claroideum*		
		C	M	N
Height (cm)	H1	20.2 a	21.2 a	22.7 a
	H2	21.8 a	23.3 a	22.5 a
	H3	24.0 a	27.2 a	27.3 a
	H4	30.5 a	31.5 a	31.1 a
	H5	32.0 a	30.2a	32.7 a
Root lenght (cm)	H1	16.8 b	19.8 b	26.0 a
	H2	13.0 b	20.2 a	23.3 a
	H3	18.4 b	26.8 a	23.3 ab
	H4	15.5 b	24.7 a	19.3 ab
	H5	19.0 b	35.0 a	35.7 a
Shoot dry weight (mg plant^{-1})	H1	17.8 c	23.2 b	27.6 a
	H2	23.1 b	28.9 a	30.8 a
	H3	26.5 c	38.9 b	42.2 a
	H4	41.0 b	44.1 a	46.5 a
	H5	55.6 b	81.8 a	83.7 a
Root dry weigth (mg plant^{-1})	H1	5.7 b	5.7 b	8.3 a
	H2	6.6 b	9.6 ab	11.9 a
	H3	10.4 b	10.8 b	14.5 a
	H4	13.2 a	15.6 a	16.0 a
	H5	15.4 b	23.3 a	24.5 a

Values in each row not sharing a letter in common differ significantly according to the Duncan test (P≤0.05).

that Myconate has little or no potassium fertilizing effect at the low concentration used, we conclude that the early and quicker mycorrhization process was responsible for the mostly significant increase in particular of root length and root and shoot dry matter formation of the plant. The shoot:root ratio was also wider (Tab. 2) which is often a clear indicator for the activity of mycorrhiza (SMITH et al., 2011). The results also indicate that the stimulation of mycorrhiza by formononetin with the consequent growth enhancement can take place even when the root infection intensity is low and below or near 1 % only.

It was clear from the results that the lignates/humates (B, P) and mixtures of humates with algae extracts (D) had little stimulating effects on the mycorrhizal development. It may be that the one single measurement of mycorrhiza parameter at 15 DAT was too early in the experiment, or that the dose rate of the products was not adequate and too high. Obviously was the highest dose of P inhibiting the entry point formation and doubtless that in particular the root dry matter production was increased over the control at the highest dose of these NP. It may be, but needs confirmation, that this effect was caused by the potassium which was applied with the humates, as well as with the Myconate. Dispher Algum contains some extracts of algae, and such extracts are known to have plant growth stimulating hormonal effects (ÖRDÖG et al., 2004; NORRIE and KEATHLEY, 2006; KHAN et al., 2009). It was striking that dry matter production at the lower doses of this product was significantly better than in the control and some of the other products. We assume that this increase was due to other effects than to mycorrhiza.

Of the two inorganics the dolomitic limestone (N) stimulated the mycorrhization of the plants, and significantly over time. Shoot and root dry weight was also higher which may have been an effect of the better and quicker mycorrhization process. The effect of N on the mycorrhiza formation cannot easily be explained. It is unlikely that direct nutritional aspects (Ca, Mg) have played any role in the growth improvement as the rates were far too low (2.5 kg ha^{-1} in the second experiment). Neutralization effects of toxic elements by the limestone can also be excluded as the soil used in the experiment was

not very acidic. It is possible that this product had an indirect effect on mycorrhiza formation by influencing positively other soil micro-organisms in the rhizosphere of wheat seedlings as the experiment was not under strict sterile conditions. It was frequently shown that helper bacteria can stimulate the mycorrhizal infection processes (JOHANSSON et al., 2004; MIRANSARI, 2011), and it may be that such micro-organisms stimulated mycorrhizal propagule germination and hyphal branching in soil as the number of mycorrhizal infection entry points was almost as high as with myconate. It is known since longer time that rhizobacteria can produce plant hormones and hormone type products like salicylic acids (MEYER and HÖFTE, 1997). Latter not only can induce resistance against pathogens in plants but also can influence positively the plant physiology and plant growth (HAYAT et al., 2007). More research is required on this subject as such effects must be powerful as they resulted in significant better plant establishment during the first 29 days after seed treatment. The silicate based product (Q) had no effect on the mycorrhization process but surprisingly the root dry matter production was significantly better that in none treated wheat. Silicates are reported to strengthen the plant tissue against attacks by pathogens or to form physical barriers against soil fungi on roots (SHEN et al., 2010), but it appears here that it did not inhibit mycorrhiza infection ratings more than other products, even at high dose rates.

Natural products, when applied to seed of plants, may have a number of different effects on the early development of mycorrhiza and plants. Some natural products like formononetin and substances in sea weed extracts may mimic flavonoids which are exudated by plant roots (STEINKELLNER et al., 2007). Such substances can be important signaling compounds for the establishment of microb-plant interactions, like arbuscular mycorrhiza (CATFORD et al., 2006). Indirect effects of NP on other soil micro-organisms are also possible, and these rhizosphere micro-organisms can produce phytohormone like substances (e.g. oligosaccharides, salicylic acids) which influence plant growth (TSAVKELOVA et al., 2006) without stimulating mycorrhiza.

The concentration of NP used for seed treatment appears to play a role for promoting the mycorrhizal infection process. In general, lower rates are more effective than higher rates. It is unclear whether this has something to do with phytotoxic effects; the seed germination (Fig. 1) was numerically slightly inhibited by some NP (D, M, Q) but not to levels which were seen critical for wheat establishment. Whether or not the initial positive effect of e.g. Myconate and Natural Green, on mycorrhiza will translate to more wheat yield, can only be found in field experiments.

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References

ALARCÓN, C., CUENCA, G., 2005: Arbuscular mycorrhizal in coastal sand dunes of the Paraguaná, Península Venezuela. Mycorrhiza 16, 1-9.

BURROWS, R., AHMED, I., 2007: Fungicide seed treatments minimally affect arbuscular-mycorrhizal fungal (AMF) colonization of selected vegetable crops. J. Biol. Sci. 7, 417-420.

CASTILLO, C.G., BORIE, F., GODOY, R., RUBIO, R., SIEVERDING, E., 2006: Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile. J. Appl. Bot. Food Qual. 80, 40-47.

CASTILLO, C.G., RUBIO, R., BORIE, F., SIEVERDING, E., 2010: Diversity of arbuscular mycorrhizal fungi in horticultural production systems of Southern Chile. J. Soil Sci. Plant Nutr. 10, 407-413.

CASTILLO, C.G., PUCCIO, F., MORALES, D., BORIE, F., SIEVERDING, E., 2012: Early arbuscular mycorrhiza colonization of wheat, barley and oats in Andosols of Southern Chile. J. Soil Sci. Plant Nutr. 12, 511-524.

CASTILLO, C.G., MORALES, A., RUBIO, R., BAREA, J.M., BORIE, F., 2013: Interactions between native arbuscular mycorrhizal fungi and phosphate solubilizing fungi and their effect to improve plant development and fruit production by *Capsicum annuum* L. Afr. J. Microbiol. Res. 7, 3331-3340.

CATFORD, J.G., STAEHELIN, C., LAROSE, G., PICHE, Y., VIERHEILIG, H., 2006: Systemically suppressed isoflavonoids and their stimulating effects on nodulation and mycorrhization in alfalfa split-root systems. Plant Soil 285, 257-266.

COVACEVICH, F., ECHEVERRÍA, H.E., AGUIRREZABAL, L.A.N., 2007: Soil available phosphorus status determines indigenous mycorrhizal colonization of field and glashouse-grown spring wheat from Argentina. Applied Soil Ecology 35, 1-9.

DAVIES, F., CALDERON, C., HUAMAN, Z., GÓMEZ, R., 2005: Influence of a flavonoid (formononetin) on mycorrhizal activity and potato crop productivity in the highlands of Peru. Sci. Hortic.-Amsterdam 106, 318-329.

GARCÍA-GARRIDO, J.M., LENDZEMO, V., CASTELLANOS-MORALES, V., STEINKELLNER, S., VIERHEILIG, H., 2009: Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. Mycorrhiza 19, 449-459.

HAYAT, S., ALI, B., AHMAD, A., 2007: Salicylic acid: biosynthesis, metabolism and physiological role in plants. In: Hayat, S., Ahmad, A. (eds.), Salicylic Acid – a Plant Hormone, 1-14. Springer, Hamburg.

ISTA, 2004: Uniformity in seed testing. International Seed Testing Association (ISTA), Zürich, Switzerland.

ISTA, 2008: International rules for seed testing. International Seed Testing Association (ISTA), Zürich, Switzerland.

JOHANSSON, J., PAUL, L., FINLAY, R., 2004: Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol. Ecol. 48, 1-13.

KAEWCHAI, S., SOYTONG, K., HYDE, K.D., 2009: Mycofungicides and fungal biofertilizers. Fungal Divers. 38, 25-50.

KHAN, W., RAYIRATH, U., SUBRAMANIAN, S., JITHESH, M., RAYORATH, P., MARK, D., CRITCHLEY, A., CRAIGIE, J., NORRIE, J., PRITHIVIRAJ, B., 2009: Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. 28, 386-399.

KÖNIG, S., WUBET, T., DORMANN, C., HEMPEL, S., RENKER, C., BUSCOT, F., 2010: TaqMan Real-time PCR assays to assess mycorrhizal responses to field manipulation of grassland biodiversity: effects of soil characteristics, plant species richness and functional traits. Appl. Envion Microbiol. 76, 3765-3775.

KOSKE, R.E., TESSIER, B., 1983: A convenient, permanent slide mounting medium. Mycol. Soc. Amer. Newslett. 34, 59.

MEYER DE, G., HÖFTE, M., 1997: Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87, 588-593.

MIRANSARI, M., 2011: Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl. Microbiol. Biot. 89, 917-930.

MORA, M.L., ALFARO, M.A., JARVIS, S.C., DEMANET, R., CARTES, P., 2006: Soil aluminium availability in Andisols of Southern Chile and its effect on forage production and animal metabolism. Soil Use Manage. 22, 95-101.

MURILLO-WILLIAMS, A., PEDERSEN, P., 2008: Arbuscular mycorrhizal colonization response to three seed-applied fungicides. Agron. J. 100, 795-800.

NAIR, M.G., SAFIR, G.R., SIQUEIRA, J.O., 1991: Isolation and identification of vesicular-arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. Appl. Environ. Microbiol. 57, 434-439.

NORRIE, J., KEATHLEY, J.P., 2006: Benefits of *Ascophyllum nodosum* marine-plant extract applications to 'Thompson seedless' grape production. Acta Hortic. 727, 243-247.

NOVAIS, C.B., SIQUEIRA, J.O., 2009: Formononetin application on colonization and sporulation of arbuscular mycorrhizal fungi in *Brachiaria*. Pesq. Agropec. Bras. 44, 496-502.

ÖRDÖG, V., STIRK, W.A., VAN STADEN, J., NOVAK, O., SORNAD, M., 2004: Endogenous cytokinins in the three genera of microalgae from the Chlorophyta. J. Phycol. 40; 88-95.

SALVIOLI, A., ZOUARI, I., CHALOT, M., BONFANTE, P., 2012: The arbuscular mycorrhizal status has an impact on the transcriptome profile and amino acid composition of tomato fruit. BCM Plant Biol. 12, 44.

SHEN, G.H., XUE, Q.H., TANG, M., CHEN, Q., WANG, L.N., DUAN, C.M., XUE, L., ZHAO, J., 2010: Inhibitory effects of potassium silicate on five soil-borne phytopathogenic fungi *in vitro*. J. Plant Dis. Protect. 117, 180-184.

SIEVERDING, E., 1991: Vesicular-arbuscular mycorrhiza management in tropical agrosystems. GTZ- Schriftenreihe N°224, Hartmut Bremer Verlag, Friedland, Germany.

SILVA-JÚNIOR, J.P., SIQUEIRA, J.O., 1997: Aplicação de formononetina sintética ao solo como estimulante da formação de micorriza no milho e na soja. Rev. Bras. Fisiol. Veg. 9, 33-39.

SIQUEIRA, J.O., SAFIR, G.R., NAIR, M.G., 1991: Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. New Phytol. 118, 87-93.

SMITH, S.E., READ, D.J., 2008: Mycorrhizal symbiosis. Academic Press, London.

SMITH, S.E., JAKOBSEN, I., GRONLUND, M., SMITH, A., 2011: Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol. 156, 1050-1057.

SPSS, 2003: SPSS® 12.0 brief guide. SPSS, Chicago, Illinois, USA.

STEINKELLNER, S., LENDZEMO, V., LANGER, I., SCHWEIGER, P., KHAOSAAD, T., TOUSSAINT, J.P., VIERHEILIG, H., 2007: Flavonoids and strigolactones in root exudates as signals in symbiotic and pathogenic plant-fungus interactions. Molecules 12, 1290-1306.

TROUVELOT, A., KOUGH, J.L., GIANINAZZI-PEARSON, V., 1986: Mesure du taux de mycorhization VA d'un systéme radiculaire. In: Gianinazzi-Pearson, V., Gianinazzi, S. (eds.), Physiological and genetical aspects of mycorrhizae, 217-221. INRA Press, Paris.

TSAO, R., PAPADOPOULOS, Y., YANG, R., YOUNG, J.C., MCRAE, K., 2006: Isoflavone profiles of red clovers and their distribution in different parts harvested at different growing stages. J. Agr. Food Chem. 54, 5797-5805.

TSAVKELOVA, E.A., KLIMOVA, S.Y., CHERDYNTSEVA, T.A., NETRUSOV, A.I., 2006: Hormones and hormone-like substances of microorganisms: a review. Appl. Biochem. Micro. 42, 229-235.

WESTPHAL, A., SNYDER, N., XING, L., 2008: Effects of inoculations with mycorrhizal fungi of soilless potting mixes during transplant production on watermelon growth and early fruit yield. HortScience 43, 354-360.

WHIPPS, J.M., 2004: Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can. J. Bot. 82, 1198-1227.

ZADOCK, J.C., CHANG, T.T., KONZAK, C.F., 1974: A decimal code for growth stages of cereals. Weed Res. 14, 415-421.

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